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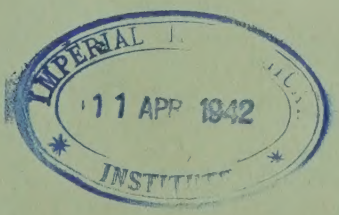


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THE BOTANICAL REVIEW

VOL. II OCTOBER, 1936 No. 10

# THE CHEMISTRY AND PHYSIOLOGY OF THE PECTINS

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## INTRODUCTION

The pectins are a heterogeneous group of carbohydrate-like, colloidal substances of extremely wide distribution in plant cell walls. Their existence has been known for many years, in fact, since 1824, when Payen (56) and Braconnot (11) described "gelée végétale" and "le principe gélatineux des fruits," substances which we now realize must have been pectins. These two workers dealt only with pectins which they could obtain in solution outside of the plant. Fremy (27, 28) recognized, however, that an insoluble pectic substance is present in cell walls, and that from this the pectins of Payen and Braconnot might be derived by boiling with water or dilute acid. This precursor of soluble pectin he called "pectose" or "protopectin." Still a third type of pectic substance was recognized by Fremy. This was obtained by heating material containing protopectin with dilute alkali. It behaved very differently from "pectin" in that it was precipitated by calcium. In addition, its solutions were acid whereas those of pectin were nearly neutral. He called this third type of substance, therefore, "pectic acid."

We have, then, three general types of pectic compounds:

1. Protopectin, insoluble in water, and found only in tissues.
2. Pectin, soluble in water, and obtained from No. 1 by acid hydrolysis; not precipitated by calcium.
3. Pectic acid, obtained from No. 1 or No. 2 by alkaline hydrolysis; precipitated by calcium.

This is the historical conception of the pectic substances. Further, it is hardly profitable to go historically in the development of our present knowledge, and I shall, therefore, rather discuss the present status with only an occasional reference to the older works.

## PECTIC ACID

Let us first consider pectic acid. The major constituent of pectic acid is galacturonic acid, or, more properly, galacturonic acid anhydride (23). Various workers have obtained various percentages of galacturonic acid in pectic acid. It may vary from 65 per cent to 95 per cent of the total. This variation is independent of worker and method and depends upon the fact that different pectic acids do really contain different proportions of galacturonic acid. The balance is made up of two substances, galactose and arabinose. The ratio of galactose to arabinose may also vary. Nanji, Paton and Ling (52) found, for example, a 1 : 1 ratio in several samples. Sloep (59) and also Ehrlich (24) have found that some pectins can be practically free of galactose, with arabinose making up one-fifth, galacturonic acid four-fifths, of the total. Ehrlich has also shown that some pectic acid may be practically arabinose-free. It is, then, galacturonic acid which is the characteristic constituent of pectic acid. Arabinose, galactose, or both may be present in varying amounts, but their quantitative relationships possess a large degree of flexibility.

The constituent molecules of pectic acid are present in the anhydride form, (*i.e.,* as in polysaccharides) and can be set free from one another by hydrolysis. It is clear, therefore, that in pectic acid itself they are linked together in some manner. For reasons which will become apparent later, they are not linked through their carboxyl groups, but rather through the usual polysaccharide linkages. There have been various schemes proposed for the manner in which this takes place. Nanji, Paton, and Ling, have, for example, proposed a "basic unit" composed of one molecule each of galactose and arabinose, with four molecules of galacturonic acid, the whole joined in a ring. Such a basic unit would, however, hardly allow for the great variation in constitution of pectic acid. Ehrlich, by continued careful hydrolysis of pectic acid, was able to obtain (a) a galactose-galacturonic acid, (b) tetra-galacturonic acids, some of which were clearly not in ring form, some of which he believed to be in the form of rings of four molecules. Since hydrolysis, Ehrlich believed that *they* formed the "basic units" of the tetra-galacturonic acids were very stable and resistant to further pectic acid and that galactose and arabinose were attached in some unspecified but loose fashion to this nucleus.

Other schemes for the structure of pectic acid have been advanced. There are, however, facts which indicate that these schemes as well as those of Nanji, Paton, and Ling, and of Ehrlich are fundamentally in error. As mentioned above, even the variable composition of pectic acid argues against the existence of such a "basic unit."

In the first place, the units of pectic acid (and of its derivatives) which are dispersed in colloidal solution are greatly elongated. The colloidal micelles are 100 times or more longer than they are wide. This elongated structure of the micelle finds its expression, for example, in the fact that when pectic acid is dried under tension in the form of threads, the micelles show a good orientation in the direction of tension. Among the markedly elongated colloidal micelles with which we are thus far acquainted, the micellar anisotropy is due to a molecular anisotropy. The best studied examples of this kind are cellulose and xylan, in which glucose and xylose, respectively, are connected by oxygen "bridges" into long chains. Moreover, these two substances are related to one another in that cellulose may be converted to xylan by removal of the sixth carbon atom of each glucose molecule. These sixth carbon atoms, as may be seen in figure 1, play no part in the main chain, and are merely "side chains." In pectic acid, one has a substance made up of galactose, arabinose and galacturonic acid, which differ from one another only in the sixth carbon atom. It seems, therefore, most reasonable to suppose that pectic acid consists of long chains of galactose molecules (Fig. 2, A), in which the sixth chain carbon atoms are free; that many of these sixth carbon atoms are oxidized to carboxyl groups (galacturonic acid, Fig. 2, B), and that some of them may be removed completely (arabinose, Fig. 2, C). Variation of the composition of pectic acid is readily understood on the basis of this structure, as is also the fact that pectin in nature is generally accompanied by greater or smaller amounts of araban (24).

The chain structure of the pectins can be verified in different ways, the most important of which is the X-ray method so successfully applied by Sponsler (62) to cellulose. Meyer and Mark (48), and particularly Van Iterson (71), have shown with this method that the galacturonic acid residues of pectins actually are arranged in chains but much work remains to be done concerning the detail

of the structure. By the X-ray diffraction method it is possible to obtain information as to the structure of the chain but not as to its length. The viscosimetric method of Staudinger (63) enables one, however, to determine the latter quantity. Henglein and Schneider (36) have applied this method to the pectins (actually to the nitropectins for technical reasons) with great success and have shown that in soluble pectin 200 or more molecules are joined in each chain.

In nature the pectic substances are always found in the isotropic state, and even in the laboratory it is difficult to cause the chains to assume even a relatively regular orientation. This, Frey-Wyssling (30) suggsts, may be due to a low order of symmetry of the pectin chains. We know that starch, which consists of glucose molecules in chains as does cellulose, crystallizes with much more difficulty than does the latter. This seems to be due partly

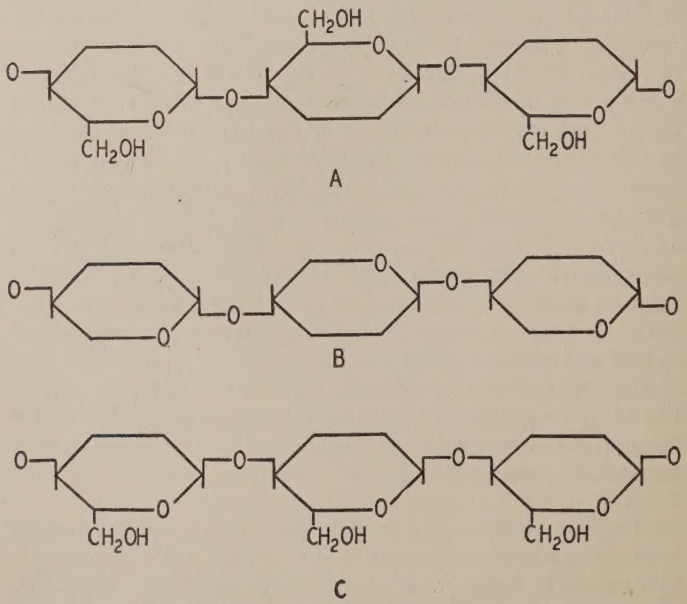


FIG. 1. Chain molecules of the glucose series:
A. Cellulose( β glucosidal linkages.
B. Xylan, β glucosidal linkages.
C. Starch, α glucosidal linkages.

to the fact that in starch the molecules are bound by α-glucosidal bridges whereas in cellulose they are bound by β-glucosidal bridges, as is shown in figure 1. By translation and rotation through 180 degrees each glucose molecule of cellulose can be superimposed upon the glucose molecule next ahead of it. In starch this is not the case, and this lower symmetry is apparently associated with a lower stability in the crystalline form. Frey-Wyssling, therefore, suggests that the chain of pectic acid, which also crystallizes with difficulty, possesses the lower grade of symmetry, as shown in figure 2.

Pectic acid does not occur as such in nature; the COOH groups are always combined in some manner or other. The combination of greatest interest is the methyl ester. All of the carboxyl groups may in theory be esterified with methyl alcohol. We would have then a pure *pectin.* In a few cases in the literature one finds pectins in which practically every carboxyl group is esterified (73, 65). In most cases, however, only a portion of the carboxyl groups are esterified and the remainder are free. It is also possible to saponify pectin by stages, and to obtain in this way a complete series of *pectinic acid,* partly methylated pectin derivatives ranging from pectin to pectic acid. We have, then, a series of compounds related to one another as follows:

| | | | |
|---|---|---|---|
| pectin | completely methylated | no free COOH groups | commercial pectins |
| pectinic acids | partly methylated | some free COOH groups | commercial pectins |
| pectic acid | not methylated | all COOH groups free | |

As we shall see later, the differences in the properties of these compounds are related principally to the difference in number of free COOH groups.

The protopectin of the primary cell wall is not directly soluble in cold water alone. If, however, it is subjected to the mildest acid hydrolysis it may immediately be extracted as pectin or a highly methylated pectinic acid. Different reasons have been adduced to account for the insolubility of protopectin. Sucharipa (65) believed pectin to be a compound of pectin with cellulose. Sloep (59), however, and also Branfoot (12), have been unable to confirm his work and at present there is certainly no positive evidence that pectin is combined with cellulose. A molecular chain structure of the pectins would make the insolubility of protopectin much more easily understood. It has been shown for a large variety

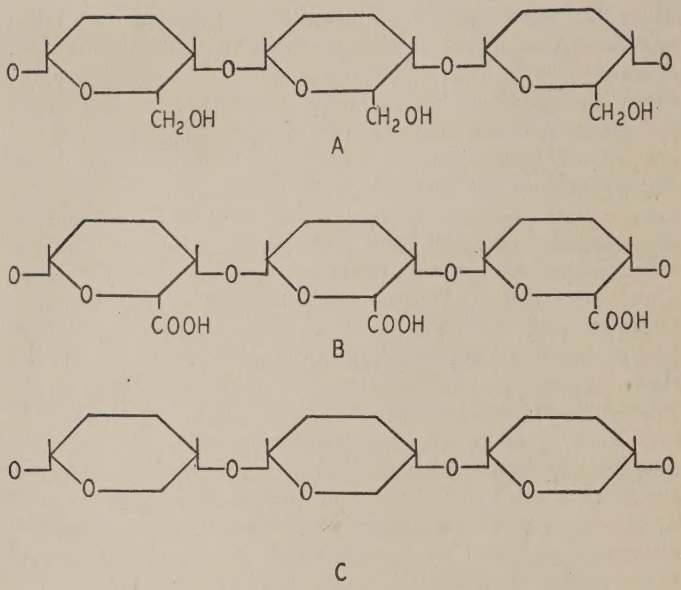


FIG. 2. Chain molecules of the galactose series:
A. Galactan.
B. Poly-galacturonic acid.
C. Araban.

of such compounds that the longer the chain the more insoluble the substance (63), and that, in addition, the longer the chain the more easily it can be split by hydrolysis, *i.e.*, the longer the chain the greater the chance that one of the links can be broken. It would seem possible, therefore, that protopectin differs from pectin in that the former is composed of longer chains. If this is the case the relatively greater ease of hydrolysis of protopectin would be at once understandable.

Let us again consider pectic acid as made up of long chains of galacturonic acid with an occasional galactose and arabinose scattered along the chain. If one adds a salt with a polyvalent cation, say $CaCl_2$, to a sol of pectic acid, one should expect an increase of "effective electrostatic attraction" (14) between the chains, and, if this attraction becomes large enough, a precipitate containing pectic acid and calcium ions. The maximal attraction is obtained

when there is one calcium ion for every two COOH groups, and if pectic acid is precipitated from a solution containing an excess of calcium such a "calcium pectate" is formed. The presence of calcium ions between chains aids greatly in orientation of the chains neatly parallel to one another, as is shown by the fact that dried calcium pectate is often double refracting.

In order to get a calcium ion to stay between two COOH groups, it is necessary to have a certain equilibrium concentration in the outside solution. If one increases the valence of the added cation this equilibrium becomes less (13) until in the case of a six valent cation it is negligible and practically every added cation is "adsorbed" on the pectic acid surfaces (7). We can make use of this fact to determine the charge density of pectic acid. If we add a very small amount of a salt having a six valent cation to a sol of pectic acid, a precipitate which is negatively charged as determined by catephoresis will appear. As one adds more of the six valent cation the charge of the precipitate becomes zero and then positive. Now suppose that we take twice the amount of pectic acid. We will find that exactly twice the amount of six valent cation is needed to neutralize all of the charges of the pectic acid, and that, therefore, practically all of the added cation has been adsorbed. From the number of equivalents of six valent cation needed to bring about zero charge of a known amount of pectic acid we know at once the number of equivalents of charge on this amount of pectic acid. This method has been applied (7) and it has been shown that pectic acid is one of the most strongly negative colloids with which we are familiar, and that, furthermore, its negative charge is principally due to its carboxyl groups. It is because of this very high negative charge density that pectic acid is so readily precipitated by electrolytes, and this fact in turn has led several investigators to describe pectic acid as "insoluble" in water, their preparations presumably containing sufficient electrolyte to prevent dispersion.

Pectinic acids differ from pectic acid as we have seen in that only a portion of the carboxyl groups are free. Correspondingly, one finds that the negative charge density of pectinic acids is lower than that of pectic acid, and that pectinic acids are less readily precipitated by electrolytes. This charge density is, moreover, dependent to some extent on the acidity of the dispersion medium, in that it becomes slightly greater in solutions of smaller acidity. Greater

charge density in alkaline solutions results in easier precipitation by electrolytes in such solutions, a fact which has been observed several times (61, 7).

The carboxyl groups of pectic acid are thus of considerable interest as the principal source of colloidal charge. We should, therefore, examine them more closely. If one makes an electrometric titration of pectic acid, one obtains a curve which resembles superficially that of a monobasic acid. However, if one now calculates the dissociation constants along the curve, one finds that they increase constantly from about pK 2.7 on the more acid side to over 4 on the less acid side (7). This variation of pK is continuous, that is, there are no "favored" ranges. We see at once that some characteristic of the pectin chain with which we are not as yet familiar, influences the strength of the carboxyl groups.

## COLLOIDAL PROPERTIES

The pectins as a group have a large number of interesting colloidal properties. It is impossible to go into these properties here in detail, and only a few will be mentioned. Ordinary pectin is readily precipitated as a solid gel by the addition of a little dehydrating agent such as alcohol. This is a rather surprising fact, since related negative colloids such as gum arabic are not so precipitated. In fact, the entire classical theory of Freundlich and Kruyt (44) was based on charge and hydration as independent stability factors. However, there is good reason for this apparent deviation of pectin from the laws of the other bio-colloids. This reason is best made clear by an analogy. If one makes up a warm solution of 1 per cent agar and allows it to cool but prevents macroscopic gel formation by very vigorous agitation, one obtains a colloidal sol of agar particles, strongly negatively charged, and stable under ordinary conditions. This sol consists, however, of microscopic and submicroscopic gel particles and the addition of a small amount of dehydrating agent brings about gelification of the entire mass. Pectin also consists apparently of very small gel particles which, under normal conditions, are not quite able to coalesce to a uniform gel. This, however, is possible if the hydration of these particles is reduced even to a small extent (see also 22).

Pectins possess another interesting property which is possibly of considerable physiological significance. They are as we have seen

easily separated from the sol by the addition of dehydrating agents. In fact, they are so sensitive to dehydration that they are readily affected by other hydrophilic colloids, that is, they are "insoluble" in many of the other bio-colloids. A sol consisting of, for example, 8 per cent pectin and 8 per cent gum arabic, separates into two layers, one of which contains principally pectin, the other principally gum arabic. If a less strongly hydrophilic colloid, such as soluble starch is chosen, it is necessary to use a larger amount of it to bring about separation of a pectin layer. This phenomenon cannot be gone into in detail here, and it is sufficient to indicate that it may be of importance in the formation of pectin structures in the cell, for example, of the layered "slimes" of cinnamon, etc. (69).

To summarize the colloidal properties of the pectins: they are hydrophilic colloids of high negative charge, a charge which varies with the number of free carboxyl groups. This high negative charge is responsible for the ready precipitation of pectic substances by electrolytes, variations in the charge causing variations in this precipitability. The pectin sols differ from typical hydrophilic sols in that they are composed of larger particles, essentially gel fragments, and this is responsible for the ready precipitation of pectins by dehydrating agents. In fact, so sensitive is the pectin sol to dehydrating agents that dehydration may even be accomplished by other hydrophilic colloids.

## DETECTION OF PECTINS

Methods for the localization of pectic substances are still relatively crude. Perhaps the best as yet discovered is the staining reagent ruthenium red (ammoniacal ruthenium oxy-chloride), introduced by that great student of the pectins, Mangin (46). This reagent stains pectins very deeply and does not stain other constituents of ordinary cell walls. It has, however, two disadvantages: *a,* it is not completely specific for pectins but rather for sixth carbon atom carboxyl groups; cellulose which has been subjected to a mild oxidation stains with ruthenium red, as do other of the "plant slimes" which contain such carboxyl groups; *b,* it does not differentiate between protopectin, pectin, and pectic acid or calcium pectate. This is a great disadvantage as will be shown below. Other methods for the microscopic localization of pectins were introduced by Mangin:

1. Removal of protopectin by hydrolysis with dilute acid, leaving the cellulose thus in place.
2. Removal of pectates, particularly calcium pectate by hydrolysis with dilute alkali.
3. Removal of cellulose with Schweizer's reagent, leaving pectins in the form of insoluble copper salts.
4. Solution of all pectic substances in warm ammonium oxalate.

By use of these methods in conjunction one can obtain a good idea of the localization of pectic substances in general, and even, more specifically, of calcium pectate. Discrimination between the different grades of pectinate is, however, impossible.

## PECTIN IN FRUITS

Protopectin occurs most richly in the primary cell wall, that is, in the nomenclature of Kerr and Bailey (39), in the two thin layers first laid down upon the two sides of the middle lamella. The walls of most parenchymous cells consist of such pectin-rich primary walls, as do the walls of meristematic cells. The pectin which is obtained commercially from apples comes to a great extent from primary cell walls of the fleshy parenchyma. The "albedo" of oranges and of lemons also consists of parenchymous cells whose primary walls are extraordinarily rich in protopectin. Protopectin is not, however, confined to the primary cell wall. Its occurrence in the secondary walls of collenchyma, for example, is common. Anderson (1) has shown that the thickened walls of the collenchymous cells in the stems of tomato consist of alternate layers of protopectin and cellulose. The outermost layer of the root hair is also said to consist of a pectic substance, although its exact nature and function is not known (57).

Protopectin may remain unaltered in the cell wall in which it is laid down. Thus Anderson (2) has shown that protopectin is formed in the primary walls of wood (black locust), and that it remains even in the old wood. It may, on the other hand, undergo marked changes. Ehrlich (24) has suggested that protopectin is converted to lignin, but this would seem improbable from the very different natures of the two substances. More likely is the suggestion that protopectin may be changed under some conditions to hemicellulose. It has been shown above that conversion of protopectin to araban, a "hemicellulose", might be expected, and Ehrlich has in fact shown that araban is always present to some extent in tissues containing protopectin. Buston (15) has also presented

evidence to show that protopectin may be changed to hemicellulose as tissues become older, and Norman and Norris (53) have converted pectin to hemicellulose-like substances by mild oxidation *in vitro*.

The most striking alteration undergone by protopectin is, however, its degradation during the ripening of fruits. This was first worked out in detail for the apple by Carré (16, 17) and Carré and Horne (19), although it had been observed both by Fremy (27) and by Mangin (46). The protopectin present in the fruit rises during maturation until a final high level is reached which is maintained for some time. During storage of the fruit the protopectin then begins to decrease and decreases rapidly for several months, less rapidly for five or more further months. This decrease of protopectin is attended by a simultaneous increase of soluble pectin, and the inference seems fair that protopectin has been converted to soluble pectin. This supposition is further supported by the finding of Carré (18) that there is an enzyme present in the apple tissue which can convert protopectin to soluble pectin. Further changes in the pectin may occur, particularly in markedly overripe fruits. The pectin is in this case hydrolyzed still further, presumably to galacturonic acid, galactose, and arabinose, in any event to substances no longer recognizable as pectin. Similar changes have been observed in the pear (19) and in citrus fruits (31).

### PECTINS IN THE MIDDLE LAMELLA

It was early recognized that pectic substances are present in the middle lamella. Thus Payen (56) regarded pectins as a sort of cement between the neighboring cells of a tissue. Mangin (46) realized particularly clearly that the middle lamella contains no cellulose but that it is rich in a pectic substance different from protopectin. This substance is not affected by boiling with dilute acid, the treatment used for removal of protopectin from the primary cell wall. It may be removed, however, by heating with dilute alkali or by precipitation of calcium present in the tissue with ammonium oxalate. Mangin, therefore, suggested that the pectin of the middle lamella is calcium pectate. That the middle lamella is actually rich in calcium was shown by Molisch (51), and that pure calcium pectate possesses solubilities *in vitro* similar to those of the middle lamella has been shown by Bonner (6, 7). The

calcium pectate nature of the middle lamella has, however, been contested by Tupper-Carey and Priestley (68), who maintained that it has the nature of a protein-pectin complex. That such complexes with protein may be formed by pectinic acids and by pectates has been shown by Bonner (7), but such complexes do not in any way resemble in their properties the middle lamella. Sloep (59) believes the middle lamella to be composed of protopectin, but, as shown above, the pectin of the middle lamella is greatly different from protopectin in its sensitivity to acid. It would seem at present that the calcium pectate theory of the middle lamella is supported by the most evidence.

The middle lamella also undergoes changes during aging of the cell wall. Carré (17) has shown that as apples become overripe there is a continuous decrease in the amount of pectic substance present in the middle lamella. At the same time the parenchymous cells become loosened or completely free from one another. The formation of intercellular spaces in general is in fact a phenomenon closely related to the pectate of the middle lamella. The surface of the cell wall abutting on the intercellular space retains a thin covering of its pectic material, and this may later be swollen into a projecting knob or wart. These have been studied in detail by Mangin (46) and by Vidal (72), as well as by Kisser (41). The knobs in the leaf spaces of *Dieffenbachia* have been carefully studied by Kisser and shown to consist of a thin tough membrane filled by a "swellable" substance. Both membrane and contents are of pectic nature, and experiments of the author have indicated that the difference between them is one of calcium content, the membrane being rich in calcium and probably approximating calcium pectate in composition, while the interior pectic substance is a pectinate very poor in calcium.

Among the commercially important processes which depend upon the dissolution of the middle lamella might be mentioned the retting of flax, which will be discussed later.

There can be no doubt as to the function of the pectates of the middle lamella, since they certainly act as a cement between the two adjacent primary walls. If this cement is removed the cells separate. It is interesting to note that calcium pectate can function also as a cement between two cellulose surfaces *in vitro*. As to the function of protopectin there is no such obvious explanation.

Since the pectins are relatively "hydrophilic" substances, it has sometimes been supposed that their presence is necessary for the hydration of young growing cell walls. Buston (15) has shown that pectins are most richly developed under conditions combining rapid growth and copious water supply. This does not of course show that the development of pectins is necessary for rapid growth, and there has been in fact no direct evidence to this effect. A widely held belief as to the function of protopectin is that it acts as a "cement" between ultra-microscopic blocks or "micelles" of cellulose. This view has recently been further developed by Farr and Eckerson (26), with the difference that they believe the cemented particles to be of microscopic dimensions. All such theories are based, however, upon the concept of the cell wall as composed of separate "bricks" of cellulose. It is now certain, however (70, 5, 30), that the cell wall is not built up in this way, but that the cellulose units or micelles are held together by cellulose molecules, and that protopectin functions only as an "intermicellar" substance, that is, that it forms its own system of long interlocking molecules in the meshes of the similar system of cellulose molecules. It seems not unlikely that the formation of poly-galacturonic acid derivatives by the cell is principally an indication of a certain level of oxidative activity in general met with only in young cells. This simple view is to some extent complicated by the above mentioned observation of Buston that the water balance of the cell also influences the formation of pectic substances. The oxidative level of the cell is again, however, related to the water content (8).

## PECTIC ENZYMES

There are at least three types of enzymes which are responsible for the interconversion and degradation of pectic substances. These distinct types have been given different names by different investigators (Table 1), but at present the widely accepted nomenclature of Davison and Willaman (21) should be used, even though this nomenclature does not agree strictly with that of the pectic substances themselves.

The first pectic enzyme is *protopectinase*. Under this classification are included: *a*, protopectinases proper, which split protopectin of the cell wall to soluble pectin; and *b*, enzymes which attack the middle lamella with resultant maceration of tissue. The second

TABLE 1

| | | | |
|---|---|---|---|
| Present Name—Protopectinase | | | |
| Names previously used | protopectinase Sloep | pectosase Carré (Branfoot) | pectosinase Bourquelot and Herissey |
| Present Name—Middle Lamella Protopectinase | | | |
| Names previously used | protopectinase Sloep<br>Ehrlich<br>Davison and Willaman | pectinase Paton<br>Jones<br>Harter and Weimer | pectosinase Beijerinck and van Delden |
| Present Name—Pectinase | | | |
| Names previously used | pectinase Bourquelot and Herissey<br>Sloep<br>Carré (Branfoot)<br>Davison and Willaman | pectolase (?) Ehrlich | |
| Present Name—Pectase | | | |
| No other name in use. | | | |

pectic enzyme is *pectinase,* which hydrolyzes soluble pectin, pectic acid, or pectates, to galactose, arabinose, and galacturonic acid. Lastly, there is *pectase,* which attacks only soluble pectin and pectinates, splitting off methyl alcohol, and forming pectic acid.

Protopectinase was first discovered by Bourquelot and Herissey (9) as an enzyme capable of converting protopectin to soluble pectin. Carré (18) has more recently shown definitely that such an enzyme is present in apple and in rutabaga tissue, and has shown that soluble pectin is the product of its activity. She also (12) stresses the difference between this true protopectinase and the enzymes of group *b* which destroy the middle lamella. It is not known, in general, whether the end product from the action of this type of enzyme is pectin, pectic acid, or degradation products as in the case of pectinase. Furthermore, it has often been found as in the case of Harter and Weimer (34) that enzymes of this class attack the middle lamella but leave the primary wall untouched. Until the middle lamella-destroying enzyme has been further studied it is, therefore, desirable to differentiate it sharply from true protopectinase.

Enzymes attacking the middle lamella are most commonly met with among the phyto-pathogenic fungi and bacteria and undoubtedly play a rôle in the maceration of plant tissue by these organisms

(37, 38, 35). In addition to the enzymatic attack of tissues there may be, however, an action of organic acids or of oxalates produced by the organism (60). Middle lamella protopectinase is found also in pollen, and Paton (55) suggests that its presence there is necessary for the penetration of the pollen tube between the cells of stylar tissue. The abscission of leaves is possibly due to a middle lamella-protopectinase mechanism (58, 59). The softening of fruits during the ripening process is undoubtedly in part a result of middle lamella-protopectinase action. Sloep has shown that in the case of fruits of *Mespilus,* ripening is accompanied by enzymatic dissolution of the middle lamella and consequent softening of the tissues.

Bourquelot and Herissey (10) also were the first to demonstrate the existence of pectinase. Their enzyme, from germinating barley, split calcium pectate to reducing substances, which we now know to have been galactose, arabinose and galacturonic acid, and later investigators have used the estimation of reducing sugars as a quantitative measure of pectinase activity. Qualitative or semi-quantitative determination can, however, be made by estimation of the time necessary to "liquefy" a gel of calcium pectate. Pectinase splits, however, not only calcium pectate but also pectin and pectinic acids, and it is, therefore, presumably present in overripe fruits, the pectin of which is hydrolyzed to galacturonic acid and sugars. This enzyme, also, is best known from fungi and bacteria, particularly *Rhizopus tritici* and *Sclerotinia cinerea,* studied by Davison and Willaman (21). These workers were able to effect partial separation of the middle lamella enzyme and pectinase by fractional precipitation with alcohol, and they also found that *Sclerotinia cinerea* which does not produce demonstrable middle lamella enzyme, produces, nevertheless, large amounts of pectinase. In general, however, ability of a micro-organism to attack the middle lamella is associated with the production of an enzyme acting upon calcium pectate, and supports the view that the middle lamella is of this nature (see 3). That pectinase capable of attacking calcium pectate *in vitro* should not *always* attack the middle lamella might easily be due, for example, to a longer chain length or to impurities of the native material.

Pectase, the best known of the pectic enzymes, hydrolyzes the methyl alcohol from soluble pectin with the production of pectic acid, as shown first by Von Fellenberg (74). In the presence of

traces of calcium this pectic acid sets to a gel, and for this reason pectase is commonly known as an enzyme causing gelling of pectin, even though Bertrand and Mallevre (4), Goyaud (32), Kopaczewski (43), and Mehlitz (47) have emphasized the rôle of calcium in the process. The action of pectase is essentially similar to that of the lipases, and Kertesz (40) has in fact shown that its action may be duplicated by lipase from *Ricinus* or even from the pancreas. To its lipase nature is probably due the fact that pectase is almost universally distributed in plant tissues, although no function of pectase as such has as yet been found.

### THE RÔLE OF PECTIC ENZYMES IN THE RETTING PROCESS

Pectic enzymes are of practical importance in the retting process. The retting of textiles, particularly flax, has long been known (42) to be due to a solution of intercellular pectic substances and consequent separation of fibers from the ground tissue of the plant. Winogradsky (74) moreover demonstrated that retting is due to definite micro-organisms, one of which he named *Granulobacter pectinovorum,* which "ferment" pectic substances. Beijerinck and Van Delden (3) even produced artificial retting with *Bacillus subtilis.* More modern investigations (20) have shown that high quality of the retted product is dependent upon a relatively high pectin content, and that it is essential that not only the protopectin of the fiber walls be left, but that also the middle lamellae joining the individual fibers into fiber bundles must remain intact. Eyre and Nodder (25), who have investigated the retting process in great detail, divided it into four periods. During the first period soluble sugars are fermented by the mixture of micro-organisms present, and considerable carbon dioxide is evolved. During the second stage some pectin is attacked by pectinases with the production of organic acids in the retting solution, but no solution of the middle lamellae is apparent. In the third stage a rapid decomposition of the middle lamellae sets in, particularly of the cortical cells surrounding the fiber bundles. Considerable quantities of organic acids, presumably galacturonic acid, appear in the retting solution. During the fourth stage the pectic substances of the fiber bundles themselves are attacked, with consequent decreases in strength and quality of the fiber. It is, then, necessary to stop the ret after the completion of the third stage, or after about 190 hours under the

conditions of Eyre and Nodder. Several pectic enzymes must apparently take part in the retting process. During the second stage pectinase appears to be the chief agent, attacking soluble pectins of the tissue. During the third stage a middle lamella enzyme as well as pectinase appear to be active, and during the fourth stage a true protopectinase attacking protopectin of the fibers is probably added to these. It is possible that the acids formed in the retting solution by the metabolism of the micro-organisms also contribute to the degradation.

### PECTINS IN JAMS AND JELLIES

By far the most important use of the pectins, aside from plant functions, is their part in the making of jams and jellies. There is a very voluminous literature on this practical subject and there is in addition no satisfactory, unified theory of the jelly formation. A short summary of the principal factors must, therefore, suffice.

It is necessary to emphasize the fact that the calcium pectate gel and the pectin sugar jelly are fundamentally different. The former is obtained when calcium ions are added to a sol of pectic acid or of a soluble pectate, and cannot be made with pectin itself. The latter is never obtained with pectate but only with pectin and certain pectinates. After the discovery of the pectin sugar jelly by Braconnot (11), its properties were studied by Fremy (28, 29) (who misinterpreted the jelly as pectic acid), Von Fellenberg (74), and Sucharipa (64) (among many others), and it was generally recognized that there are three principal factors involved in jelly formation: pectin, sugar, and acid. For the formation of a good jelly, pectin and sugar in suitable concentrations must be brought together at the appropriate acidity. The temperature at which this is effected is of some importance but as a matter of convenience, the boiling point of the solution is often used. Excessive heating, however, reduces the strength of the jelly.

Protopectin is incapable of forming sugar jellies, and for this reason green fruits containing much protopectin and little pectin are not suitable for jelly-making unless the protopectin is first hydrolyzed by boiling with dilute acid. Since pectic acid does not form a sugar jelly, it is also necessary to avoid alkaline solutions as well as the action of pectase from the fruit juice. As to the amount of pectin necessary, this varies greatly with different preparations

and modes of treatment of the protopectin. With a good pectin it can be as little as two tenths of one per cent in the final jelly, with a poor pectin as much as one and one half per cent. The properties of pectin which affect its jellying power were first studied extensively by Von Fellenberg (74). He suggested that high jellying power depends upon high methyl alcohol content, and this view has been widely accepted. Lüers and Lockmüller (45) for example found that pectins with a methoxy content of less than seven and three tenths per cent do not jell at all while those with a methoxy content of ten to twelve per cent jell very well. Meyers and Baker (49, 50) have, however, reinvestigated this question and have shown that the parallelism is probably secondary and merely accompanies other changes in the pectin which are of more direct importance. Decreasing methoxy content is accompanied by decreasing viscosity of the pectin sol (74) and viscosity is, as shown by Meyers and Baker, definitely related to jellying power. With decrease of viscosity of a given pectin (by boiling for different times) a parallel decrease of jellying power is brought about, and this relation holds from low viscosities to relative viscosities of about fifteen. Above this point increase of viscosity is not attended by any increase in jellying power. Among different samples of pectin the relation does not hold so well, due apparently to variations in galactose-arabinose content of the pectin. Removal of galactose and arabinose increases jellying power but decreases viscosity. From this work of Meyers and Baker we can, however, definitely say that jellying power does depend upon "degree of polymerization" of the pectin, that is, upon the length of the pectin chains.

The amount of sugar used in preparation of the jelly may vary within wide limits, depending on the grade of pectin, the pectin concentration, and the hydrogen-ion concentration. The concentration in the final jelly is much more nearly constant and is approximately saturated (sixty-five to seventy per cent), even though with very pure pectin. Tarr and Baker (67) have been able to prepare jellies without the addition of sugar. The ratio of pectin to sugar is of great importance in the determination of the strength and texture of the jelly. High sugar-pectin ratios lead to a soft jelly; low ratios to a tough jelly. The particular ratio needed depends, moreover, on the "degree of polymerization" of the pectin used, that is, the longer the pectin chains the more sugar in relation to pectin is

necessary for the production of a soft jelly. If pectin of constant chain length is used, then the higher the concentration of pectin the firmer the jelly. The ratio of pectin to sugar necessary for the production of a jelly of a given strength also decreases as the hydrogen-ion concentration decreases from pH 3.4 to 3.1 (67).

The sugar factor in the pectin-sugar jelly is then rather complex and depends upon many variables. Although it is commonly believed that sugar acts only as a dehydrating agent for the pectin (see 54), still the existence and significance of these variables are not as yet adequately explained.

The presence of acid has long been known to be essential for jelly formation, but Tarr (66) first showed that it is not the quantity of acid but the acidity (pH) which is the controlling factor. The optimal pH of jelly formation is in the region 3.2 to 3. Acidities below this give weak jellies, above this, "syneresis" or expressing of liquid. With increasing pectin concentration the optimal pH shifts slightly downward, which it does also with increasing sugar concentration. Some pectins possess sufficient free acid groups to bring the jellying mixture to the correct pH, and in such cases the addition of more acid is unnecessary. Natural fruit juices also often contain sufficient acid. With fruit juices which possess sufficient pectin and sugar but insufficient acid, such as certain peaches and pears, jelly formation can be brought about by the introduction of acid. The function of acid is also not well understood although it has been proposed that it has principally to do with reduction of the pectin's negative charge, thus making approximation of the pectin units easier (59). If this should be the case, one would expect that other highly charged cations should have a similar effect. Critical experiments have not as yet been made, but the investigations of Von Fellenberg and of Halliday and Bailey (33) indicate that calcium may at least partially take the place of hydrogen-ion. It is well known that the jellying power of natural fruit juices varies greatly. Juice from overripe fruit fails to jell since the pectin has been destroyed by the action of pectinase. Green fruit or fruit rich in protopectin but poor in pectin demands long cooking in slightly acid solution to bring about hydrolysis of protopectin to pectin. In some cases (green apples, lemons, some grapes) the fruit itself contains sufficient acid to bring about this hydrolysis, in others acid must

be added (peaches, pears, figs, melons). Rhubarb on the other hand contains sufficient acid but insufficient pectin of any kind, and strawberries are deficient in both acid and pectin.

In conclusion it might be remarked that detailed instructions for the preparation of jellies from several different fruits may be found in a special report of the Delaware Agriculture Experiment Station.

## CONCLUSION

It has been attempted to show that the pectic substances as a group are of interest from the most varied points of view, and that all these points of view have contributed to our present knowledge. The structure and the colloidal behavior of the pectic substances are of interest to the chemist. In industry pectins are met with, either as filtering problems or as essential components of industrial products. But the pectins are, after all, plant products of the greatest interest to the botanist and to the physiologist who study their manifold functions in the plant, and the relation of chemical structure to these functions. From what has been said in the foregoing pages it is apparent, however, that for a complete understanding of pectic substances, much remains to be done, by both chemist and botanist.

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# THE PRESENT STATUS OF SEED TREATMENT, WITH SPECIAL REFERENCE TO CEREALS

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Man's unceasing struggle against the destructive forces of Nature is well illustrated by his war on plant diseases. Losses due to fungous and bacterial diseases of commercial crops in the United States amount to about a billion dollars annually (107). The annual loss caused by one disease, bunt of wheat, may amount to over 25 million dollars (38). During the 11-year period 1917–1927 smuts in oats caused an average annual loss of 50,000,000 bushels (119). These losses combined with those caused by other diseases of these and other crops, make a staggering total.

Much of this loss is preventable. The principal preventive measures include sanitation, crop rotation, and certain other cultural practices, the use of resistant varieties, and seed treatment.

The purpose of this paper is to sketch briefly the development of seed treatment, especially for cereals, and to describe the present problems, trends, and practices in that field as they apply to conditions in the United States.

No attempt will be made to review more than a small part of the prodigious amount written on the subject, or even to cite more than a small percentage of the articles perused. Some of the statements made are based on the writer's own experiments, data from which have not been published.

## HISTORICAL

Seed treatment for the prevention of plant diseases, especially those of cereals, has been practiced, in one form or another, for about three centuries.

At first, in the absence of definite knowledge concerning the nature of plant diseases, preventive measures were of a more or less superstitious nature, such as sowing in the dark of the moon, or sticking branches of laurel in the grainfields "to draw the blighting vapors to them" (136). Some of the earlier materials used for treating cereal seeds were lime, salt, saltpeter, and wood ashes.

Copper sulfate probably was the first standard fungicide used, and its intelligent application dates back to 1761 (113). It did not come into general use, however, until a century later when Kühn's (65) experiments established a basis for making definite recommendations regarding its use. Later investigators made other recommendations concerning the use of copper sulfate, the most important of which was that, after treatment, the grain be dipped in lime-water to prevent seed injury. Despite the advances made in seed treatment methods in recent years, many farmers still use the copper sulfate treatment for the control of bunt in wheat.

Another seed treatment method of early origin still in use is the hot-water treatment developed by Jensen (51) in 1887. It still is the only known treatment that will kill certain deep-seated fungi like that causing the loose smut of wheat (*Ustilago tritici*) which are not controlled by surface disinfectants. It also is used for treating certain vegetable seeds.

Formaldehyde was first advocated as a seed treatment in Germany by Geuther (32) in 1895 and in the United States by Bolley (6) in 1897. It still ranks among the foremost liquid treatments because of its cheapness and its general effectiveness, in spite of its tendency to injure the seed.

Copper sulfate and formaldehyde continued as the outstanding seed treatment materials up to about 1914. Mercuric chloride and other materials were tried but not generally recommended. In 1912 organic mercury compounds were introduced as seed disinfectants in Germany and in early experiments Riehm (104, 105) along with others found them effective in cereal-disease control. Among the first of these to be marketed was a chlorophenol mercury compound known as "Uspulun", placed on the market in Germany about 1915. Similar compounds under the trade names "Chlorophol" and "Semesan" soon appeared in the United States. These materials were used in solutions ranging in concentration usually from .25 to .75 percent. In general they were found very effective in cereal-disease control with little or no seed injury (68, 71, 106, 122, 126).

Dust disinfectants first came into prominence as a result of the work of Darnell-Smith with copper carbonate (18, 19) in Australia in 1915. Due to certain apparent advantages this form of seed treatment met with immediate popularity and started the era

of dust fungicides. At first the use of dust fungicides was restricted to the control of diseases due to surface-borne organisms such as bunt of wheat; but experiments soon showed that the more deep-seated organisms, like those causing the smuts of oats (27) and covered smut and stripe of barley (73), could be reached by certain dust fungicides (69, 71, 72). From then on, liquid fungicides lost favor and dust fungicides gained in popularity, not only for treating cereal seeds but seeds of other crops. Although liquid fungicides have by no means been entirely discarded, yet if dust fungicides of equal effectiveness can be economically used, they usually are preferred.

### ADVANTAGES OF DUST FUNGICIDES

Dust fungicides possess certain outstanding advantages over liquid fungicides.

1. They are more easily applied; the wet disagreeable task incidental to immersion treatments is eliminated as also is the work of drying the seed, and the danger of the seed freezing, sprouting, heating or moulding before it dries.

2. The use of dust fungicides greatly reduces the chances of errors in seed treatment. Liquid fungicides frequently are used in excessive concentrations or the immersion period may be too long and thus impair germination. On the other hand the solution may be too dilute or the period of immersion too short to effect disease control. Certain fungicidal solutions when used repeatedly have so much of their essential ingredients taken up by the seed that additional concentrated solution must be added occasionally (28, 64). Mistakes are easily possible in adding the chemical to replenish the solution.

It also is possible to apply too much or too little of a dust fungicide, but such errors are revealed to some extent by the appearance of the treated seed. The excess dust usually is readily discernible, while if an insufficient amount is applied its failure to coat the seed properly will be evident especially if the recommended rate per bushel is 2 ounces or more. Dusts that give off fumes and thus disinfect the seed largely before it is planted, must be applied with greater precision as to amount than dusts that are relatively inert until the soil moisture acts upon them.

3. Dust fungicides furnish greater protection against recontamination of the seed.

4. They protect the seed, to some extent, against soil organisms.

5. They protect the seed against weevils and rodents (79).

6. Their application is largely independent of temperature while low temperatures decrease the effectiveness of some liquid fungicides (31, 66, 79).

Dust fungicides are not without certain disadvantages. The fine dust when inhaled may cause extreme discomfort or even illness. The vesicant action of mercurials is especially disagreeable. With the exception of formaldehyde, liquid fungicides usually do not affect the operators while being applied.

Dust fungicides may interfere with the ready flow of grain through the drill, especially those dusts applied at the rate of 2 or more ounces per bushel of seed. Conners (13), for example, found that treatment with copper carbonate increased the bulk of wheat seed 7.7 percent and reduced its rate of flow about 12 percent. Insufficient or excess soil moisture (131) or the presence of certain organic materials in the soil (115, 116) may decrease the effectiveness of some dusts that do not take effect until after sowing.

Dust fungicides, as a rule, are more expensive than liquid disinfectants.

### PROBLEMS IN DEVELOPING AND TESTING SEED TREATMENTS

The work of testing the relative effectiveness and practicability of different fungicidal materials presents certain problems to the investigator. The first of these relates to obtaining a supply of seeds carrying sufficient infective material to provide an adequate test for the fungicide. If the organisms are entirely surface-borne, as in bunt and flag smut of wheat, and kernel smut of sorghum, it is a simple matter to infest the seed. But in many cases the organisms are placed by Nature deeper within the seed (27, 73), in a manner that cannot be easily and surely duplicated artificially. The disparity in results obtained by different investigators may sometimes be explained by the fact that in one case naturally inoculated seed was used and in the other the seed was artificially inoculated. This makes it desirable to secure seed from a badly infected crop, or still better, if possible, seed from

the same lot from which the infected crop was grown. In some years, strange as it may seem, badly infected fields are not readily found. In other years, even though the infection may be high in the field, the weather or other conditions may not be conducive to a heavy invasion of the seed by the organisms (70). Such seed when used in seed treatment experiments yields disappointing results because it fails to test thoroughly the merits of the fungicides under study. Again, suitable seed may be available, but the conditions under which it germinates or the plants are grown may not favor development of the disease; thus another experiment may end inconclusively.

Effectiveness in disease control, however, is only one of the factors by which the relative practicability of a seed disinfectant is judged. We also must consider its "chemotherapeutic index" or margin of safety (31); its effect upon germination, stand, vigor, and yield; its corrosive effects on treating and sowing machinery (25); its injurious effects on the persons applying it or handling the treated seed; its stability, cost, and other features. For example, fungicidal dusts containing a considerable percentage of such materials as mercuric chloride or mercuric iodide are undesirable because of their corrosiveness and extremely poisonous nature. Many effective dusts have proved commercially impracticable because their high mercury content made the price prohibitive. The chief objection to certain volatile dusts is their rapid deterioration unless they are kept tightly sealed. Many effective materials, such as copper chloride, are too hygroscopic to retain the fluffiness desirable in a dust fungicide; others, like cuprous oxide, are not always chemically stable but gradually change to other forms unless specially treated.

A comparison between the large number of materials that have been reported effective in plant-disease control from time to time by various investigators and the relatively few commercially successful fungicides now available for effectively controlling these same diseases, seems to indicate that most of the substances found effective in an experimental way were found unsuitable in the manufacture of practicable fungicides, either because of undesirable physical or chemical properties or excessive cost. The production of experimental seed disinfectants was, for a time, a popular "side line" of many commercial concerns. The realization of the

fact that, like Rome, good practicable seed disinfectants cannot be built in a day, has resulted in fewer of these products being put on the market in recent years.

### CEREAL DISEASES COMBATTED BY SEED TREATMENT

The different diseases that attack cereals will not be described here and only those will be mentioned that are wholly or in part amenable to control by seed treatment. Adequate descriptions of these different diseases will be found in a number of publications (8, 34, 40, 60, 61, 62, 77, 85, 120).

Some of these diseases, such as barley stripe, are associated with a single host; others, such as scab, are common to several cereal hosts.

General recommendations for seed treatments of wheat, oats, barley and grain sorghums in the United States are very briefly outlined in a recent Federal publication (52). More detailed information on diseases or materials and methods for their control is available in other publications (4, 5, 7, 8, 16, 34, 35, 41, 61, 62, 77, 85, 89, 127, 134).

Wheat probably has received more study from the standpoint of seed-borne diseases and their control than has any other cereal. Orton (92) lists over 50 organisms that have been isolated from wheat seed by various investigators. Henry (39) isolated species of fungi representing between 15 and 20 genera. Among these the most common were species of *Alternaria, Fusarium,* and *Helminthosporium.* In recent studies on light-weight wheat the writer isolated species of *Alternaria, Helminthosporium, Fusarium, Penicillium, Aspergillus, Rhizopus, Macrosporium, Cladosporium,* and bacteria from the seeds. Most of these have been isolated also from other cereal seeds. The presence of these organisms on seeds does not necessarily indicate that they are pathogenic. Most of them, however, are at least capable of attacking the food material in the germinating seed and thus preventing or inhibiting germination. To some extent this can be prevented by seed treatment, a fact that applies to the other cereal seeds as much as it does to wheat.

The principal seed-borne diseases of wheat, known to be wholly or partly amenable to control by seed treatment, are: bunt (*Tilletia tritici* and *T. levis*), flag smut (*Urocystis tritici*), loose smut

(*Ustilago tritici*), seedling blights, caused by certain species of *Fusarium* and *Helminthosporium,* black chaff (*Bacterium translucens* var. *undulosum*), basal glume rot (*Bacterium atrofaciens*) and anthracnose (*Colletotrichum graminicolum*).

Loose smut can be controlled only by the hot-water treatment, which, if applied, also eliminates the other seed-borne diseases. The latter, however, insofar as they are caused by seed-borne organisms, generally respond to treatment by the better organic mercury compounds, such as ethyl mercuric phosphate. Formaldehyde, although not used so widely as formerly, also is effective, but, at times, its benefits do not compensate for the injury it causes. The better copper dusts, such as copper carbonate, basic copper sulfate, and copper oxychloride will prevent bunt and flag smut (20) in the absence of soil infestation, and will partially control some of the other diseases mentioned. They also afford some protection against soil-borne organisms, but are less effective in this respect than the better organic mercurials.

Rye is somewhat susceptible to some of the diseases mentioned under wheat. Among these are bunt, loose smut, anthracnose, and the seedling blights caused by species of *Fusarium* and *Helminthosporium.* Rye also is attacked by stem smut (*Urocystis occulta*), and by bacterial blight caused by *Bacterium translucens* var. *secalis.* None of these diseases, except possibly stem smut, is serious in the United States, and seed treatment of rye is not generally practiced. In Europe the disease known as snow mold "Schneeschimmel" (*Fusarium nivale*) is very important and generally is combatted with organic mecurials. The treatments recommended for wheat also may be used for rye.

The important seed-borne diseases of barley are: covered smut (*Ustilago hordei*), brown loose smut (*U. nuda*), black loose smut[1] (*U. nigra*), stripe (*Helminthosporium gramineum*), anthracnose (*Colletotrichum graminicolum*), bacterial blight (*Bacterium translucens*), and the seedling blight stages of net blotch (*Helminthosporium teres*), spot blotch (*H. sativum*), and scab (*Fusarium graminearum*). As in the case of wheat, the hot-water treatment is the only one that prevents brown loose smut (*U. nuda*). The better organic mercurials, such as ethyl mercuric phosphate, are most effective against the others, especially stripe. Formaldehyde

[1] Also called by some false loose smut or intermediate loose smut.

in liquid or dust form will control the covered and black loose smuts fairly well and also will reduce considerably the amount of seedling infection caused by other seed-borne organisms. Copper dusts, on the whole, are relatively ineffective against barley diseases except in those varieties having hull-less seeds.

The seed-borne diseases that affect oats are: covered smut (*Ustilago avenae*), loose smut (*U. levis*), leaf spot (*Helminthosporium avenae*), anthracnose (*Colletotrichum graminicolum*), halo blight (*Bacterium coronofaciens*), bacterial stripe blight (*B. striafaciens*) and the Fusarium seedling blights caused by such organisms as *F. graminearum, F. culmorum, F. avenaceum,* and *F. nivale.* Organic mercurials, such as ethyl mercuric phosphate and others (90, 137), formaldehyde liquid, and formaldehyde dust disinfectants, are widely used for disinfecting oat seed. They are named above in the order of preference. As in the case of barley, copper dusts are effective only on the seed of hull-less varieties.

The name sorghum, used in its broadest sense, includes groups known as broomcorn, darso, durra, feterita, hegari, kafir, kaoliang, milo, sorgo, sweet or saccharine sorghum, and Sudan grass. There also are numerous crosses of the above groups. The most common seed-borne diseases of sorghum are covered kernel smut (*Sphacelotheca sorghi*) and loose kernel smut (*S. cruenta*). Head smut (*Sorosporium reilianum*) usually is brought about by soil infestation, but it is possible that the seed may carry the disease into new areas (92). Anthracnose (*Colletotrichum fulcatum*) also may be carried on the seed, although, like head smut, this is not the chief way in which it is spread. Certain bacterial diseases also are suspected of being seed-borne, chief of these being bacterial streak (*Bacterium holcicola*), bacterial stripe (*B. andropogoni*), and bacterial spot (*B. holci*). Seedling blights and seed rots frequently are caused by species of *Penicillium, Fusarium, Aspergillus, Rhizopus* and other genera borne on the seed or present in the soil.

In inoculation experiments with a number of organisms of the above genera, *Penicillum oxalicum* was found capable of causing an especially severe seedling blight of sorghum, and *Fusarium culmorum* attacked the seed and prevented germination.

The smuts and other diseases of sorghum insofar as they are seed-borne can be prevented by the organic mercurials previously mentioned, copper carbonate, sulfur, and other common surface disinfectants in dust form. Formaldehyde and copper sulfate solutions are no longer recommended because of the danger of seed injury. (21, 22, 55). Losses from seed rots and seedling blights, caused largely by soil organisms, can be greatly reduced by copper and organic mercury dusts. The damage to seed caused by these organisms varies with soil conditions and with the thickness of the seed coat (117).

Proso and foxtail millets also are attacked by sorghum kernel smuts in addition to smuts peculiar to these two grasses, (*Ustilago panici-miliacei* and *U. crameri,* respectively). The treatments recommended for sorghum are suitable also for the millets.

Rice is a cereal in the culture of which seed treatment plays a very minor part. Most of the important rice diseases are soil-borne, and seed treatment has little or no effect on their incidence. The hot-water treatment has been recommended for seedling blight caused by species of *Helminthosporium* (77, 125) and a modified mercuric chloride treatment for seed-borne diseases of rice in general (75). Tullis (letter) states that seed-treatment with cuprous oxide and formaldehyde reduces the number of seedlings infected with *Curvularia lunata* and that 2 percent ethyl mercuric chloride reduced infection by *Helminthosporium oryzae.* However, he stated that no seed treatment was wholly effective against soil-borne organisms, and none caused significant increases in yield, and, therefore, seed treatment of rice is not recommended as a practical farm procedure in the United States.

Flax is subject to a number of diseases that are caused by soil-borne organisms and, therefore, not controlled by seed treatment. Chief among these is wilt (*Fusarium lini*). The pasmo disease (*Phlyctaena linicola*), anthracnose (*Colletotrichum linicolum*) and browning or stem-break (*Polyspora lini*) may be seed-borne, although all are largely spread by other agencies. Various seedling blight and damping-off organisms also may be seed-borne.

Seed treatment of flax has not been widely practiced. The recent literature on the subject has been reviewed by Flor (23). The latter in 4 years' experiments found seed treatment of flax with copper carbonate, organic mercurials, and formaldehyde did

not benefit consistently either stand or yield. These results are not in agreement with others obtained in Iowa (10, 101) where considerable increases in yield followed the use of disinfectants containing ethyl mercuric chloride and phosphate. Flor (23) attributes this disparity in results to differences in climatic factors and soil flora in North Dakota and Iowa.

The chief seed or seedling diseases of corn combatted by seed treatment are those caused by *Diplodia zeae, Gibberella saubinetii, Basisporium gallarum, Fusarium moniliforme, Penicillium oxalicum, Aspergillus niger, A. flavus,* and other species of *Penicillium, Rhizopus,* and *Fusarium* (43, 63). Orton (92) names organisms representing 20 genera that may be seed-borne but some of which cannot be controlled by seed treatment. Only seedling diseases of corn are successfully combatted by seed treatment (61). Other diseases such as ear rots, smut, stalk rots, leaf blight, root rots, and bacterial wilt are not eliminated by seed treatment.

Different investigators are not entirely agreed regarding the advisability of treating seed corn every year. This is probably on account of differences in soil and climatic conditions in different parts of the country. In certain experiments in Kansas (82), Arkansas (81), and Nebraska (58, 59), no consistent benefits followed the use of seed disinfectants for corn, while in certain other States farther east and north seed treatment proved beneficial and is recommended as a good farm practice (43, 63, 84, 98, 100, 102, 103).

In extensive seed-treatment studies made in connection with the Iowa corn yield test in 1933, 1934, and 1935 (108, 109, 110), seed treatments frequently improved stands, but significant yield increases were obtained only from hybrid seed, and then only in certain sections and in certain years. In no case did seed treatment of open-pollinated seed cause significant increases in yield. This seed, it is thought, may have been more carefully selected and hence of better quality than the hybrid seed.

In general it has been found that good corn seed that is found relatively free from disease-producing organisms when tested in the seed germinator, is not benefited by seed treatment unless sown in cold wet soil. In the latter case, good fungicidal dusts protect the seed to some extent from soil inhabiting organisms.

Seed, infected to any considerable degree with the organisms mentioned above, especially *Diplodia zeae,* usually is benefited by seed treatment and particularly if cold wet weather follows planting. Much of the injury due to soil-borne organisms can be reduced by proper cultural practices (63). Corn seed treatment is not a substitute for careful seed selection and seed testing, but may be regarded in the same light as crop insurance. Whether or not benefits will accrue from corn seed treatment frequently depends upon factors or conditions that vary from year to year and are beyond the control of the grower.

The liquid fungicides formerly recommended for treating seed corn have been discarded in favor of fungicides in dust form. Some of the earlier dust treatments, such as Sterocide, Bayer Dust and others, have already become obsolete. The current treatments most frequently suggested in recent investigations are New Improved Semesan Jr. (successor to Semesan Jr. and Improved Semesan Jr. but changed in composition), Barbak 111, and Merko. All of these contain mercury. Copper fungicides on the whole have been found unsatisfactory (82) as corn seed treatments.

### FUNGICIDAL MATERIALS

The foregoing discussion of the principal diseases of cereals amenable to control by seed treatment may well be followed by a brief appraisal of the fungicides generally used for their control. These materials may be arranged into several general groups:

1. Non-metallic substances such as formaldehyde and related materials, including formaldehyde dusts, paraformaldehyde dusts, and mixtures of formaldehyde with other materials, sulfur, hot water, hot water and alcohol, calcium and sodium compounds, phenols, etc.

2. Copper compounds, mostly inorganic, such as copper carbonate, sulfate, oxide, chloride, oxychloride, acetate, oxalate, phosphate, arsenate, and others.

3. Mercury-containing compounds, mostly organic, but also some inorganic.

4. Salts of other metals, such as zinc, nickel, lead, etc.

Formaldehyde (liquid) used as a soak, dip, sprinkle, or spray still retains considerable popularity as a fungicide. In cereals it is used mostly for the control of the smuts of oats, and bunt of

spring wheat, although it also is used to some extent for treating barley, sorghum, and other seeds. For bunt control it is best used as an immersion treatment, so that the bunt balls, light kernels, and trash can be skimmed off. After immersion in a 1:320 solution the wet seed is drained, covered for several hours, and then spread out to dry sufficiently to be run through the drill. If the seed is cleaned so that it contains no bunt balls, other methods of application may be used. The spray method is the most popular for oats (37). One pint of formaldehyde diluted with 1 of water is sprayed on 50 bushels of oats as the latter are shoveled over. The sprayed oats are covered from 5 to 8 hours and then sown, no drying being necessary. In this, as in all other treatments with formaldehyde, the seed should be sown as soon as possible after treatment to prevent injury.

The chief advantages of formaldehyde are its cheapness and its general effectiveness if properly applied. Unlike many other fungicides, it does not render the seed unfit for feed and this is a decided advantage. Its great disadvantage is its narrow margin of safety and the many factors that make it injurious to the seed (49). It not only fails to protect the seed against soil organisms but makes it more susceptible to them (76). Frequently, although it eliminates a considerable percentage of disease from a crop, it reduces the yield below that obtained from untreated seed.

Formaldehyde dust was designed to make available for seed treatment the fungicidal effectiveness of formaldehyde without its attendant disadvantages (111, 112). It consists of an inert material, like talc, charcoal, kaolin or infusorial earth, which has been allowed to absorb 4 to 8 per cent by weight of commercial formaldehyde. The finished product must be kept tightly sealed to have it remain effective. It is used chiefly to combat the smuts of oats (*Ustilago avenae* and *U. levis*) and, to some extent, covered smut (*U. hordei*) and black loose smut (*U. nigra*) of barley. However, since it does not control barley stripe (73) it is not generally recommended for that cereal. It is applied to grain at the rate of about 3 ounces per bushel, after which the grain is sacked and allowed to stand for several hours or overnight. Prolonged storage after treatment may impair somewhat the viability of the seed. The extent of this injury varies with different varieties of grain and also with the moisture content of the seed,

the relative atmospheric humidity, and the conditions under which the seed is stored.

Oats or barley may be treated with formaldehyde dust for about 3 to 5 cents per bushel. This dust is easily applied, it usually causes no serious injury if the treated seed is stored properly and not too long, it does not seriously affect persons applying it and it does not render the treated seed wholly unfit for feed.

Its greatest disadvantage is its rapid deterioration when it is exposed to the air and the resulting disappointing results frequently obtained with it. As a rule, it does not significantly increase yields from clean seed and occasionally depresses yield (62, 74, 137). Since the formaldehyde spray effectively controls oat smut, is easily applied, and is much more dependable and cheaper, there is little argument for the use of formaldehyde dust for this purpose. It fails to control stripe in barley and, therefore, cannot be considered an ideal material for treating that cereal. It is not effective in bunt control and is not recommended for sorghum. It satisfactorily prevents damping-off and other seedling diseases of certain vegetables and ornamentals (1), and is now widely used in combatting them (133).

Paraformaldehyde occasionally has been tried as a fungicide, but with little success. Recently, the writer obtained promising results in oat-smut control with Formacide, a recently developed paraformaldehyde dust. According to the manufacturers, this dust contains a catalytic agent that, in the presence of moisture, causes the paraformaldehyde to revert to the gaseous formaldehyde. One of its apparent disadvantages is that treated seed cannot be stored a long time without injury. Further experiments with this material are necessary to determine its possibilities and limitations. Mixtures of liquid formaldehyde with mercuric chloride and other materials, as a rule, have not proved satisfactory for seed treatment.

Sulfur has been recommended as a promising fungicide for the control of covered kernel smuts of sorghum and closely related crops (55, 83, 129). Its use also has been suggested for the control of barley covered smut (56, 87). In experiments by the writer it was ineffective in controlling smuts of wheat (68), oats, or barley. It is possible that a relatively high temperature may increase the fungicidal effectiveness of sulfur by causing it to

volatilize more rapidly (93). This would make it more effective as a seed disinfectant if applied during very hot weather, although Horsfall's (45) experiments indicate otherwise. Its fungicidal properties are demonstrated by its control of cereal rusts (78), powdery mildew, potato bacterial wilt and other diseases. It is cheap and relatively harmless to seed, machines, and operators. If there could be found an oxidizing or other agent that would increase its effectiveness in seed treatment (67, 121), it would be a valuable addition to the present supply of cereal seed disinfectants.

The hot-water method of treating seed (29, 30, 36, 42, 91, 95, 118, 130) is not widely advocated because of the tediousness of applying it and its narrow margin of safety. However, it remains the only practicable means of controlling the loose smuts of wheat (*Ustilago tritici*) and barley (*U. nuda*), and is employed occasionally when smut-free seed of these cereals is not available. Usually, only enough seed is treated to sow a seed plot that will supply smut-free seed the following year. Attempts have been made with varied success to lower the temperature and lessen the time of immersion necessary for loose smut control by adding about 3 percent alcohol to the water (29, 30, 91). Frequently, the hot-water treatment seems to lower the percentage of germination, especially if the seed is tested shortly after treatment. Germination, however, may be better several weeks after the seed has been treated. Alpha barley, for example, germinated 63 percent 2 days after the hot-water treatment had been applied and 83 percent two weeks later. Untreated seed of the same lot germinated 90 percent. It has been found that copper carbonate sometimes improves the germination of wheat previously subjected to the hot-water treatment (36).

Copper and mercury appear to be the chief metals employed in seed disinfectants. Certain salts of nickel, lead, and zinc have been found effective at times in the control of some cereal diseases but have never been widely utilized for that purpose. At present zinc oxide is being advocated to combat post-emergence damping-off (46) in vegetables and ornamentals. For this purpose it is applied to the soil rather than to the seed. It also has been used successfully, however, as a seed disinfectant for spinach and tomato seed (14, 15).

The numerous papers published in recent years on the rôle of copper carbonate as a seed fungicide should make its use as such familiar to anyone interested in seed treatment. It is used principally to control bunt of wheat and the kernel smuts of sorghum. It also is effective for treating millet, flax, hull-less oats and hull-less barley, although it generally is not recommended for the three latter cereals.

There are two grades of copper carbonate dust disinfectant on the market in the United States. The better grade contains about 50 per cent metallic copper in the form of equal parts of copper carbonate and copper hydroxide. The cheaper grade contains from 18 to 25 per cent metallic copper, mostly as copper carbonate and copper hydroxide, but frequently with some copper sulfate, calcium sulfate, or other ingredients. The better grade usually is sold as "copper carbonate," but dusts made up of the cheaper grade frequently are sold under trade names such as: Cupro-Jabonite, Coppercarb, Smut-Bane, and others.

In general, experience has shown that when seed wheat carries a rather heavy spore load, the better grade of copper carbonate is more effective in bunt control than are the dilute brands, applied at the same rate. When the pure and dilute grades are employed at 2 and 3 ounces per bushel, respectively, they are about equally effective. On the whole, the better grade is preferable both for wheat and for sorghum.

The chief advantages of copper carbonate are its relative cheapness—the cost of treating wheat ranging from 2 to 4 cents per bushel—and its harmlessness to seed germination.

Its disadvantages are its tendency to cause injury to the drill and illness to the operators, and its ineffectiveness under certain soil conditions (115, 116, 128).

Copper sulfate in solution, although still used to a limited extent, is no longer recommended for cereals because it is tedious and disagreeable to apply and frequently is injurious to the seed. As formerly applied and, to some extent, still used, a pound of copper sulfate and a pound of common salt (sodium chloride) are dissolved in from 5 to 10 gallons of water, the grain is immersed in this solution, drained, dipped in milk of lime and then spread out to dry. The milk of lime is made by slaking 1 pound of quicklime (calcium oxide) and then adding enough water to make 10 gallons.

The reaction of the two solutions coats the seed with a basic copper compound that is more or less insoluble and serves to protect the seed, to some extent, from soil infestation.

Copper sulfate in powdered form has been found almost equal in effectiveness to copper carbonate in bunt control (80). Its outstanding disadvantage is its tendency to lose its finely powdered form and consolidate more or less into lumps (80, 129). This prevents it from adhering to the seed sufficiently to be effective as a dust fungicide. If the common penthydrated form ($CuSO_4 5H_2O$) is changed by heat to the monohydrated ($CuSO_4 H_2O$) or anhydrous form ($CuSO_4$), it retains its powdered form for a longer time and is more easily applied as a dust disinfectant.

Basic copper sulfate has been found by the writer and by others (115) to be more effective and cheaper in bunt control than most brands of copper carbonate. Unlike copper sulfate (bluestone), it retains its fluffy powdered form about as well as does copper carbonate. It is an especially effective preventive for bunt of wheat, and also may be used for other cereals where copper carbonate has been found satisfactory.

Other compounds of copper have been found effective in the control of certain cereal diseases by the writer and by others (86, 94, 114, 116, 123, 128), but few have reached the stage of profitable commercial production. Copper oxychloride, effective in bunt control, has been marketed but not widely sold in the United States. Copper oxalate, acetate, phosphate, silicate and other less common copper salts have effectively controlled bunt in several years' experiments by the writer, but they possess no particular qualifications that enable them to compete successfully with copper carbonate and other fungicides already firmly established on the market.

Cuprous oxide, now widely sold as a dust fungicide for vegetable seed (47), also was found by the writer to be effective for bunt control but has not been widely used for that purpose.

### ORGANIC MERCURIALS

There are at present for sale in the United States relatively few fungicides containing organic mercury compounds, although during the past 15 years a considerable number of such materials have been tried in seed treatment investigations (72, 73, 102, 106, 126,

132). Frequently it has been found that the percentage of mercury necessary to make a fungicide effective also makes its cost prohibitive and, therefore, its production unprofitable. The first organic mercury fungicides were used in liquid form. Among these were such products as Chlorophol, Semesan,[2] Seed-O-San, and variously numbered "Bayer," "Corona," "DuPont" and other compounds (126). Along with these were tried various materials of foreign make such as Uspulun, Germisan, Tillantin, and others not commercially available in the United States. With the advent of dust fungicides, experiments with these liquid treatments were largely discontinued and gave way to work on experimental dusts, many of which bore labels similar to those on the earlier products used in solution. Most of these failed to attain commercial success either because of lack of consistent effectiveness, seed injury, or excessive cost. Among the first mercury dusts used to any considerable extent were such materials as—Bayer Dust (hydroxymercurinitrophenol sulphate), Sterocide (mercury furfuramid), Semesan Jr. (hydroxymercuricresol), Semesan (hydroxymercurichlorophenol sulphate), and Merko (3.5 per cent metallic mercury in inert filler).

Some of these materials (Bayer dust, Sterocide) are no longer being made, while others have been considerably changed in composition. Among the better-known mercury dusts now on the market are the "Dubay" products. These are made by the Bayer-Semesan Company, which concern combines the former activities of two other concerns along this line.

Ceresan,[3] 2 per cent ethyl mercuric chloride, one of the first "Dubay" products, was at first recommended for controlling certain diseases of wheat, oats, barley, sorghum, and flax, and, on the whole, proved effective for that purpose. However, the recommended application of 2 to 3 ounces per bushel made its high cost —9 to 14 cents per bushel of seed—an impediment to its widespread use for cereal-seed treatment. It is now recommended as a treatment for cotton seed (88, 135), peas, and seeds of some ornamentals.

New Improved Ceresan, another "Dubay" product, is now widely used for cereal-seed treatment. It contains 5 per cent ethyl

[2] A product by this name is still being marketed.

[3] The "Ceresan" made in Germany contains phenyl mercuric acetate as its essential ingredient.

mercuric phosphate and is applied to wheat, oats, barley, sorghum, and flax at the rate of only ½ ounce per bushel at least one day before sowing. It is a so-called volatile dust and much of the disinfection is done before the seed is sown. However, if sown not more than a few days after being treated, the seed carries with it into the soil a certain amount of protection against certain soil-inhabiting organisms that frequently attack germinating seeds and unemerged seedlings. The longer sowing is delayed after treating the seed with New Improved Ceresan the less protection is furnished against these organisms, because more of the volatile fungicidal material will have been dissipated. On the other hand, prolonged post-treatment storage of the seed is most effective against the seed-borne organisms. Under certain conditions, however, prolonged storage may impair slightly the germinative capacity of treated seed.

Another Dubay dust, New Improved Semesan, Jr., is now widely used for treating seed corn. It contains 1 per cent of ethyl mercuric phosphate as its toxic ingredient. Dungan, *et al.*,[4] in extensive field tests with composite lots of seed corn from local farms in Illinois, in 1935, found that this material improved germination and stand, and increased the average yield of sound corn 9 per cent. In the same series of tests the yield from seed treated with Barbak 111 was 3 per cent greater than the yield from untreated seed. Barbak 111 contains 10 per cent mercuric phenyl cyanamide and 5 per cent lead diethyl dithiophosphate. It is advertised solely as a disinfectant for seed corn. Merko, 3.5 per cent metallic mercury in inert material, has compared favorably with other materials in some experiments (44, 84). Excellent results followed the use of these materials in experiments in Iowa (98).

## CONTROL OF DISEASES OF VEGETABLES, ORNAMENTALS, AND OTHER CROPS

The control of diseases affecting vegetables and ornamentals is a more complicated matter than that of those affecting cereals because one has to deal with a much greater variety of hosts and parasites. Many of these diseases are soil-borne or spread by wind, insects, or other agencies and do not respond to seed treatment. Methods of control in these cases are restricted largely to soil disinfection, if

[4] Unpublished data.

practicable, crop rotation, sanitation, spraying and dusting or the use of resistant varieties. In growing vegetables and ornamentals a greater variety of cultural practices also is involved. Some seeds, for example, are planted directly in the field, while others are planted in flats or hotbeds and the seedlings later transplanted. The latter method involves operations and problems foreign to cereal culture.

Prevention of damping-off is one of the chief problems in growing vegetables and ornamentals. This trouble is largely caused by species of *Pythium, Rhizoctonia,* and *Fusarium.* There are two phases of the disease, the pre-emergence and post-emergence stages. Soil sterilization by steam or by chemicals, particularly in flats or beds for transplants, frequently will control the former but not the latter phase of this disease.

Formaldehyde dust has been widely recommended as a soil disinfectant for damping-off prevention (1, 124, 133) and has met with much success. Recently cuprous oxide applied to the seed has been advocated for the control of damping-off (47) and growers of vegetables and ornamentals have found it very effective, especially for the pre-emergence stage of the disease. Organic mercurials also have been used with some success.

The use of zinc oxide as a soil-surface disinfectant for the prevention of the post-emergence phase of damping-off has met with considerable success (46). In this respect it is not used as a substitute for cuprous oxide but in conjunction with it. It also may be used as a fungicide to combat the earlier phase of the disease but with few exceptions, cuprous oxide is reported to be more effective in this respect (46). However, zinc oxide along with zinc hydroxide incorporated in the proprietary product Vasco 4 was found most effective in Virginia for treating seed of spinach and tomatoes (14, 15). Other materials recommended for soil treatment to combat soil-borne organisms are mercuric chloride solution, calomel, potassium, sodium or calcium nitrate, lime, calcium cyanamide, sulfur, sodium tetraborate, various organic mercurials, acetic acid, ammonium sulfate, and a number of other substances.

The recommendations for controlling seed-borne diseases of vegetables and ornamentals are so many and varied that they will not be reviewed here in detail. For many vegetables, such as the crucifers, tomatoes, onions, and peppers, mercuric chloride still

figures prominently in seed-treatment recommendations. Others specify organic mercurials in liquid or dust form, hot water, calcium hypochlorite, and various other treatments. There are many publications dealing with the general subject of diseases of vegetables and ornamentals and their control (11, 12, 17, 33, 57, 96, 97, 99, 112, 133), in which may be found generally accepted, although not always the most recently developed, methods of seed treatment.

*Forage Crops.*—Seeds of forage crops have not figured very prominently in seed treatment experiments according to the literature on the subject. One objection to the fungicidal treatment of seeds of legumes is its possible effect on the nitrogen-fixing bacteria with which the seeds subsequently may be inoculated (2). Horsfall (45), in his study of meadow-crop diseases and their control, makes no definite seed-treatment recommendations, and there seems to be a paucity of information in the literature upon which to base definite recommendations of that kind. Buckholtz (9) improved seedling stands of alfalfa, *Dalea, Lespedeza,* and several clover varieties by treating the seed with an organic mercury dust before sowing in *Pythium*-infested soil, but alfalfa seedling stands were depressed in *Pythium*-free soils.

*Cotton.*—The treatment of cotton seed with fungicides is a comparatively recent development. Delinting with sulfuric acid, while effective in controlling bacterial blight and probably some other seed-borne diseases, is sometimes responsible for poor stands especially in cold wet soil. It also is a somewhat cumbersome and dangerous operation (88). Hydrochloric acid gas (88) also may be used, but treatment with this requires special equipment and is practicable only on a commercial scale. Ceresan (ethyl mercuric chloride) and other fungicidal dusts have been found beneficial by several investigators (3, 135) and are more easily applied than the other two materials.

As the result of 8 years' experiments with fungicides for cotton seed at State College, Mississippi, L. E. Miles[5] recommends Ceresan, iodine dust, cuprous oxide, and zinc oxide as the most promising in their effects on germination and yield. The amount of benefit to be derived, he states, depends greatly upon the condition of the seed—age, viability, and seed-borne organisms—the condition of the soil, and the date of planting.

[5] Correspondence.

*Tobacco.*—Recommendations for treating tobacco seed have not been materially changed during the past 15 years. The control of tobacco diseases is more a matter of soil sterilization, sanitation, and cultural practices than seed treatment (53). Mercuric chloride probably is the most widely used disinfectant for tobacco seed. The seed is soaked for 15 minutes in a 1–1000 solution, then thoroughly washed and dried (26). Immersion in a 1–1000 solution of silver nitrate for 15 minutes (54) followed by repeated washing also is recommended. Formaldehyde (26) also is used but is not as satisfactory because it is somewhat less effective and is more likely to cause seed injury.

Organic mercurials have not been sufficiently tested in tobacco seed treatment experiments to merit recommendation for that purpose.

*Sugar beets.*[6]—Damping-off is one of the most troublesome diseases encountered in growing sugar beets. It may be caused by the seed-borne organism *Phoma betae* and also by certain soil-inhabiting species of *Rhizoctonia, Pythium* and *Aphanomyces.* A seed treatment to be wholly effective, therefore, not only would have to disinfect the seed but also would have to protect it and the young seedling from the attack of these soil-borne organisms. Dust fungicides, on the whole, have been found more nearly effective and more practical for this purpose than liquid fungicides, largely because of the residual protection they afford the seed after planting. Borax, copper carbonate, cuprous oxide, formaldehyde dusts and certain proprietary preparations such as Ceresan, Germisan, Agrosan and others have been used with varied results for combatting beet diseases and for improving stands. Other materials have been applied to the soil to protect the seedling during and after emergence. Among these may be mentioned copper sulfate, zinc oxide, sodium nitrate, formaldehyde, cyanide compounds, ammonium sulfate, ammonium hydroxide, boric acid and sodium borate.

The relative effectiveness of a large number of fungicidal materials has been studied in extensive field and greenhouse experi-

[6] The above discussion of beet-seed treatment was prepared with the kind assistance of Dr. G. H. Coons, principal pathologist in the Division of Sugar Plant Investigations. The information was obtained largely from material being prepared for publication as a U. S. Department of Agricultural Technical Bulletin by G. H. Coons, J. E. Kotila, E. L. LeClerg, J. G. Lill, and S. B. Nuckols.

ments carried on by workers in the United States Department of Agriculture for a number of years. The most effective fungicidal dusts developed by these workers contain both mercuric chloride and copper carbonate, the latter serving both as a diluent and as a slowly soluble or residual fungicide. Strikingly beneficial results have been obtained in greenhouse and small field trials, but large-scale tests have revealed a very complex situation which needs further study and analysis before the treatment of sugar-beet seed can take its place as a routine farm practice. The results of beet-seed treatment are influenced not only by environmental conditions but also by the type of pathogen predominating in the soil. The preceding crop also may be an important factor. Certain crops seem to augment the number of pathogenic soil organisms; other crops apparently repress them. With the aid of a rotation system employing desirable crop sequences, and the improvement of soil conditions by drainage and the application of fertilizers, it is very likely that the proper treatment of sugar-beet seed will result in securing satisfactory stands under all but the most unfavorable conditions.

### CENTRALIZED SEED TREATMENT

In the earlier stages of its development, seed treatment was entirely a farm operation carried out on the farm by the farmer. The increase in the loose smuts of wheat and barley in certain sections of the country shortly after the war created a demand for seed that had been subjected to the hot-water treatment. Since this treatment is not easily carried out on the average farm, central treating stations were established in certain localities by energetic county agents and others, and these proved very successful (95). This lesson in centralized seed treatment was remembered after dust treatments became popular, and custom treating has become the practice in many localities. Millers or elevator operators who have installed efficient large-scale treating outfits charge from 3 to 6 cents per bushel for treating the farmers' seed grain. The disadvantage of this arrangement is the trouble involved in hauling the grain to and from the central treating unit.

This objection to custom treating is overcome by the use of portable or itinerant cleaning and treating outfits. These are compactly made and mounted on trucks. They go from farm to farm,

thus saving the farmer the trouble of hauling his seed to a central plant. The charge is about 4 to 8 cents per bushel for cleaning and $2\frac{1}{2}$ to 5 cents for treating. Outstanding examples of this type of outfit are Keck Gonnerman "Kay Gee" cleaner-treaters operated in Indiana, and the "Discreenair" service in California.

Some seedsmen are making it a practice to treat seed of wheat, oats, and barley before they sell it to the farmers. T. W. Wood and Sons, of Richmond, Va., The Eastern States Farmers' Exchange, Springfield, Mass., and the Grange League Federation, Buffalo, N. Y., are pioneers in this direction. The Richmond concern at first restricted its activities in this field to dusting wheat with copper carbonate at the request of the customer. All wheat, oats, and barley now sold by them to the retail seed trade is first treated with ethyl mercuric phosphate (New Improved Ceresan). Several seedsmen in New York apply the dry formaldehyde treatment to seed oats for sale. Others use organic mercury dusts. Inquiries regarding approved materials and equipment for treating seed on a large scale seem to indicate a growing interest on the part of seedsmen in selling treated seed. The problem of efficiently applying the different dust fungicides to cereal seeds has been met by the development of a number of improved devices for this purpose (50).

### EFFECTS OF SEED TREATMENT

Plant stimulation from seed treatment is a subject that has received considerable investigation. It generally has been shown that any benefits derived from seed treatment can be traced to the control of organisms found either on or in the seed or in the seed bed. Occasionally, when untreated seed is sown for comparison, pronounced differences in vigor, stand, or yield are noted without any apparent indication of disease in the plants from untreated seed. These differences are then likely to be regarded as indications of stimulation. The diseases prevented or controlled may not, however, be externally evident because of lack of sporulation, *i.e.,* so-called latent infection may be present in the plant (138, 139). This has been shown to occur especially in resistant varieties. Flor *et al.* (24) demonstrated it in the case of bunt, Hubbard and Stanton (48) in oat smut, and Zade (138, 139) in several cereal diseases. Others have shown that little or no benefit was

derived from treatment of sound disease-free corn, planted under favorable soil moisture and temperature conditions (58, 59, 81, 82, 108, 109, 110). Similar results have been obtained with oats (74). On the other hand, when germination is delayed by cold, wet soil fungicidal dusts may protect the seeds and seedlings from attack by soil organisms which otherwise might attack and destroy the seed itself or invade the slowly developing seedling and either kill it or cause a weak plant of low yielding capacity.

With the extensive amount of research being conducted by commercial concerns and also by State and Federal agencies, on the development of disinfectants for the control of plant diseases, the composition of fungicides will continue to change. Materials now being widely used will either be further improved or will be replaced by other materials that will be more effective, cheaper, less harmful to the seed, or more acceptable in other respects. The constant aim will be to find or develop disinfectants that are highly toxic to parasitic fungi and bacteria but relatively harmless to the seeds and plants parasitized by them.

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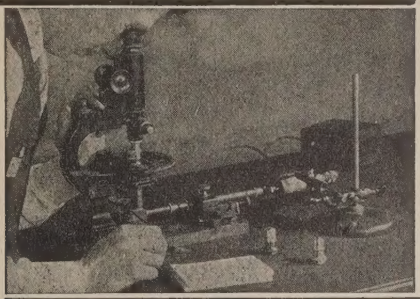

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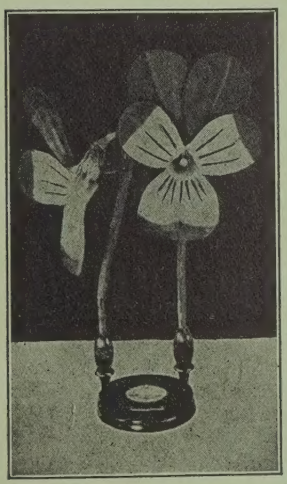

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